**Annex**

EFSA has reviewed and provided its opinion on four consecutive Monsanto monitoring reports on the cultivation of MON 810 in the EU (EFSA, 2011, 2012a, 2013, 2014). Although EFSA agrees that the conclusions of the initial risk assessment are confirmed by Monsanto's monitoring findings, further intensification in the methodology of Monsanto's Insect Resistance Management (IRM) plan is proposed. More specifically, the implementation of the 'hotspot' concept and the increased resistance allele frequency detection (1-3%) were consistently repeated and materialized progressively over the four EFSA opinions[1]. By proposing these 'improved' methodologies, we understand that EFSA's main concern is that potential resistance should be detected before field failures occur allowing timely implementation of management measures. It is our view that if the recommendations as proposed by EFSA would be implemented, this would require a significant activity increase for the consent holder in terms of insect resistance monitoring, without any obvious and proportional benefit in detecting risk to human and animal health or the environment. Our view is further substantiated below.

1. **Familiarity and experience with MON 810 support a *status quo* in terms of currently implemented IRM**

First of all, insect resistance – should it occur – rather has a negative economical impact than an adverse effect on the environment. A farmer experiencing decreased pest susceptibility will encounter plant damage and potentially negative yield effects, and is expected to complain about underperformance of the product. He will contact his distributor/retailer, who on his turn will forward the complaint to the seed company. Finally, the seed company selling the MON 810 variety will need to respond to the issue by doing an in-depth analysis of the situation, such as assessing whether indeed a MON 810 variety was grown, whether good agricultural practices were implemented and whether no other root causes were triggering an insufficient product performance. In case resistance development cannot be excluded, other additional insect management practices will be evaluated and the site will be more closely monitored.

As explained several times to the European Commission and EFSA before, this is a situation seed companies want to avoid as it would have direct impact on their business and product reputation. Therefore, high internal standards to our product stewardship efforts are set as is exemplified by Monsanto's membership to the Excellence Through Stewardship® organization[2]. A biotech seed company such as Monsanto wants to market sustainable products both from an environmental as from an economical perspective. Minimizing the probability of insect resistance to develop is a key component of such a stewardship plan for a *Bt* maize product. The lack of impact of MON 810 on the environment was discussed in the environmental risk assessment of the initial renewal application in 2007 and confirmed by EFSA in several opinions (EFSA, 2009a, 2012b).

Global experience teaches that the detection of greater than expected damage induced by the target pests are first of all found by our internal stewardship processes (farmer complaint handling and customer relations), and not through an intensification of laboratory data generation of collected larvae in the field. Unlike larvae collections, farmer complaints can theoretically be expected from all farmers using MON 810 varieties and therefore do not constitute a subsample as every MON 810 field can trigger a farmer complaint. Farmers are the first in line to notice damages and since a healthy crop producing an optimal yield is their first priority, they will seek to understand the cause of detected damage as soon as possible and consequently consult the technology provider. This approach is not limited to biotech products, such as MON 810, but implemented for all commercial products (chemistry, seed) and has a

[1] We found that these recommendations were best exemplified in the 2013 EFSA opinion, hence, this is the core document we like to address.

[2] http://excellencethroughstewardship.org/ – Accessed 20 November 2014

long validated and workable history. We are confident that these implemented stewardship plans will provide superior warning of reduced product performance when compared to a significant increase in sampling in and around MON 810 maize fields which still would remain a subsample of the total area grown.

It is in the best interest of the technology providers to implement a rigid, proportional and workable IRM plan. We believe the proposal that was originally approved in 1998 by the European Commission and is still implemented today is a proportional and workable plan. Neither the available susceptibility monitoring data nor real life commercial experience indicates a need to change the currently implemented IRM plan.

It needs to be emphasized that MON 810 maize is cultivated for approximately 15 years in the EU without any farmer complaint on its performance that was caused by resistance development, and without any scientific report or indication of reduced target pest susceptibility. On a global scale this experience is confirmed: no changes in susceptibility for neither *Ostrinia* nor *Sesamia* have been detected (Monsanto Europe S.A., 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014; Siegfried and Spencer, 2012; Siegfried *et al.*, 2007; Tabashnik *et al.*, 2013). These are clear indications that the implemented IRM strategies meet the objectives. We concur that refuge compliance is key to sustain this success. Therefore, we agree with EFSA's opinion to continue to focus on the education of farmers and encourage farmers to communicate any concern related to product efficacy. This is why besides our farmer complaint handling systems, the farmer education by training, educational leaflets and clear technical user guides (TUGs) remain a key component of our stewardship plans for the commercialization of MON 810 varieties.

Further, we challenge the drastic increase of recommended requirements in terms of monitoring for Cry1Ab-containing GM crops, whereas monitoring requirements for insecticidal crop protection products remain constant and, as a matter of fact, are practically non-existent, although their use is more widely adopted (on more acres) than MON 810 in the EU. Insecticidal crop chemicals are stewarded on a merely voluntary basis. It is perceived to be known that resistance to chemical insecticides can evolve in insect pests (see Whalon *et al.* (2008) cited in EFSA (2009b)). However, to our knowledge in the EU no monitoring conditions are imposed to any approved active substance controlling insects.

Taking a practical approach, we looked at all active substances with insecticidal activity used by Spanish farmers during the 2012 growing season. These data come from our farmer questionnaire in context of our voluntary General Surveillance plan monitoring the cultivation of MON 810 in the 2012 growing season. Farmers reported to use the following active insecticidal substances (in between brackets the number of farmers in Spain is provided who used the specific active substance): Clothianidin (166), Chlorpirifos (39), Abamectin (32), Lambda-cyhalothrin (29), Fipronil (27), Thiamethoxam (19), Deltamethrin (10), Imidacloprid (6), Thiacloprid (2), and Bifenthrin (2). Reading the appropriate legislation allowing these active ingredients to be used on the EU market as plant protection products, *i.e.*, Commission Implementing Regulation (EU) No 540/2011 (except bifenthrin, where Commission Implementing Regulation (EU) No 582/2012 is applicable), for none of the active substances active monitoring is required for target pests. In other words, no IRM plan has to be implemented by the consent holder for any of these actives. It has to be noted that most of the uses of these actives are a seed treatment providing a targeted exposure, as is the case for *Bt* crops. As mentioned before, development of resistance to traditional insecticides or to *Bt* crops is a business risk and not an environmental risk. Therefore, similar as for active substances, monitoring plans should not be required for *Bt* crops. This leads us to conclude that the current IRM monitoring conditions imposed to *Bt* crops are already non-proportional to the risk.

As a general conclusion, we challenge EFSA's recommendations to the consent holder to intensify the IRM practices since the risk of resistance development is an economic risk for which the onus is on the technology provider, and is not an environmental risk. Monsanto's rigid stewardship plans are designed to provide early warnings. To date, experience for MON 810 at the EU as well as global level demonstrate that these plans are successful in terms of preventing resistance in the European target pests ECB and MCB to develop. Furthermore, the additional efforts would be neither proportional to the (demonstrated

absence of) risk to human and animal health or the environment related to the cultivation of MON 810, nor proportional to what is required for traditional insecticides and are unlikely to detect decreased susceptibility of the insect pests earlier than with the methods currently implemented, as will be further substantiated below.

**2. The likelihood for resistance development in ECB and MCB in Europe is extremely low**

*(i) Cry1Ab resistance alleles in ECB and MCB are rare*

An effective resistance monitoring program will provide management information at a relevant time scale, and will depend on the biology of the target pest. MON 810 expresses a high dose of Cry1Ab that has a toxic effect on ECB and MCB. Apart from the suppression of the pest, the use of a high-dose product has the advantage that (by definition) in case of any resistance mechanisms developed by the pest organisms, these would be inherited in a recessive manner (Gould *et al.*, 1997; Gould *et al.*, 1995; Pereira *et al.*, 2008; Tabashnik *et al.*, 2002). Current scientific knowledge suggests that the frequency of resistance alleles in populations of ECB and MCB in Europe is low and that these alleles are recessive (Bourguet *et al.*, 2003; Gaspers, 2009), which is confirmed by previous studies. Several studies, using $F_2$ screens, conclude that Cry1Ab resistance is rare for both ECB (Andow *et al.*, 1998; Engels *et al.*, 2010; Stodola *et al.*, 2006) and MCB (Andreadis *et al.*, 2007), estimating the frequency of resistance alleles to be below $10^{-3}$. The expectation is therefore that less than one in a million ECB or MCB individuals will survive on MON 810. The likelihood of two resistant individuals surviving MON 810 can therefore be expected to be below $10^{-12}$.[3] Because of good refuge compliance in the EU and the rarity of homozygous, resistant individuals, there is a much greater probability that a resistant individual will mate with an adult from the refuge and produce offspring susceptible to MON 810.

During the years 1996 – 2005, data from approximately 2000 iso-female lines from published ECB studies have never detected resistance alleles. Using $F_2$ screen information from the published literature assessing Cry1Ab resistance in ECB, the number of iso-female lines tested in a particular geography/year ranged from 9 – 483. Andow and Alstad (1999) have estimated the need to test 750 iso-female families to have sufficient confidence that you have sampled sufficiently to detect resistance at a frequency of $10^{-3}$. The inability to test sufficient numbers of ECB families therefore generates great uncertainty in the estimation of the frequency of Cry1Ab resistance alleles. In addition, it has become increasingly more difficult to locate sufficient larvae of both species in conventional maize for resistance monitoring efforts, indicating that the numbers of adults for $F_2$ screens is also more limited. EFSA previously agreed that the $F_2$ screen is cost-prohibitive to allow efficient screening of resistance in ECB and MCB.

*(ii) MCB and ECB insects are highly mobile ensuring high gene flow and random mating*

For ECB, many studies have been conducted to determine the genetic diversity and baseline susceptibility of ECB populations to Cry1Ab. The results showed that there is a low genetic differentiation of ECB populations in Europe and no geographic clusters of populations have been detected (Chaufaux *et al.*, 2001; Farinos *et al.*, 2004; Gonzalez-Nunez *et al.*, 2000) demonstrating a high gene flow. This was also confirmed by analysis conducted with ECB in Europe by Saeglitz *et al.* (2006). Baseline susceptibility of ECB in populations collected from different EU countries showed some variability, but no consistent pattern emerging, suggesting that there is an intra-species variability in susceptibility to Cry1Ab .

Studies for MCB have also been conducted to determine the genetic diversity and baseline susceptibility to Cry1Ab (De La Poza *et al.*, 2008; Gonzalez-Nunez *et al.*, 2000). The results showed that population genetics of MCB collected from populations in Spain and southwest France were closer than populations collected from Italy, Greece and Turkey (De La Poza *et al.*, 2008), suggesting a small genetic

[3] Assuming the resistance allele frequency in male and female populations is the same and equals $10^{-3}$, the likelihood for one homozygote individual to appear is $10^{-6}$. To generate resistant (homozygote) offspring, two resistant individuals have to develop, one male and one female. The likelihood for this combined event is therefore $10^{-12}$. Taking into account that both parents have to be within flying distance of one another, the likelihood will be below $10^{-12}$.

differentiation between West Mediterranean and East Mediterranean populations. However, differences between regions only accounted for 7% of the total molecular variation (versus. 80% within populations) and no significant differences in the susceptibility to Cry1Ab were found when comparing MCB populations from these two areas (Farinos *et al.*, 2011). Moreover, MON 810 is not commercialized in East Mediterranean countries like Italy, Greece or Turkey.

In conclusion, there is no apparent difference in susceptibility of individual target pest insects towards the Cry1Ab protein across Europe. The amount of gene flow among Europe populations also confirms random mating and sufficient adult movement for an effective high-dose/refuge IRM strategy. The lack of genetic differentiation also indicates that sampling at a finer geographic scale will not add increased sensitivity to the current resistance monitoring program.

*(iii) A 20% refuge implementation in the EU is a very conservative approach*

An appropriate level of refuge should be determined based on a comparative analysis of refuge strategies and maize-growing conditions in countries where *Bt* maize is regularly cultivated. The minimum proportion of non-*Bt* refuge implemented in the US (for single mode of action) and Argentina is 20% and 10%, respectively. Such refuge sizes are considered to contain generous safeguard margins under the respective growing conditions[4] (US EPA, 2001). Therefore, EuropaBio, including Monsanto, reiterated several times that the 20% refuge implementation is a very conservative number for *Bt* maize plantings in the EU.

A comparative analysis between agricultural landscapes in the US and the EU highlights the fragmented and diverse cropping conditions in the EU. This explains why the current refuge requirement in the US of 20% is considered highly generous for the EU, thereby providing justification for a potentially lower level of refuge in the EU. Indeed, as specified in the Harmonised Insect Resistance Management Plan for Cultivation of *Bt* maize in the EU from the EU Working Group on Insect Resistance Management (2003[5]), the US corn belt maize cultivation practices can be regarded as the worst-case scenario with the highest probability to favor pest resistance to *Bt* crops. These conditions correspond to where maize cultivation and *Bt* maize adoption are greatest and insect pressure is highest. In the US, this occurs in Nebraska, Iowa, Minnesota, Illinois and Indiana. These five states routinely account for approximately 54% of the US maize crop area and represent the conditions with the highest potential for the development of insect resistance. Considering the evolution of MON 810 adoption in the EU which is currently restricted to Spain, Portugal, Czech Republic, Slovakia and Romania, these are the only ones that need to be considered for potential resistance development. Together they represent 2.3% of the EU maize cultivation area.

A comparison of these *Bt* maize-growing areas in the EU and the US indicates that there are important differences between them that make the risk of resistance development significantly lower for the EU than for the US Corn Belt. Examination of three key variables - land committed to agriculture, farm size and crop diversity – clearly demonstrates that the EU agricultural landscape is much more fragmented than that of the US, thereby favoring greater durability of *Bt* maize in the EU, and implying that the EU is leaning towards a *de facto* implementation of an Integrated Pest Management (IPM) strategy.

- The US Corn Belt has an overwhelming 50 – 89% of its land committed to agriculture, whereas the five *Bt* maize-growing EU countries have between 39 and 56% of their land committed to agriculture (Table 1).

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[4] The 20% refuge threshold was required by the US EPA for the US in 2000 following a proposal based on practical experience and studies by academia and industry. For Argentina, a 10% refuge was proposed by industry and independent entomologists based on abundant alternative hosts for the target pests, knowledge on the pest biology and grower behavior, and was subsequently accepted by the regulatory agency Comisión Nacional Asesora de Biotecnología Agropecuaria (CONABIA).

[5] This document can be found as an Appendix to the Monsanto annual monitoring reports for MON 810 cultivation in the EU which were submitted until 2012.

- Romania, Spain, Portugal, Slovakia and Czech Republic have an average of four times more farms per unit of farmland compared to the US Corn Belt (Table 1). A greater number of farms will result in increased crop diversity in an area. The fragmentation increases the probability that small maize fields will be bordered by other crops, weedy/grass barriers or fallow land.
- Maize is the major crop of the US Corn Belt and constitutes 22 to 46% of the total agricultural land and only slightly less of the total land area. However, maize constitutes only 2 to 29% of the total agricultural land of EU Member States.
- Non-maize cereal crops cover approximately 21, 23, 1.3, 27 and 35% of the arable land in Romania, Spain, Portugal, Slovakia and Czech Republic, respectively. Other important crops such as sunflower, potato and sugar beet also serve as alternate hosts for *O. nubilalis* (Hodgeson, 1928) (Table 2). These alternate hosts provide additional refuge area for the insect pests, which are not accounted for in the 20% refuge requirement for farmers, which makes the structured refuge requirement inherently conservative.

Furthermore, it needs to be recognized that the compliance to refuge implementation is very good in the EU. The farmers in the Czech Republic, Romania and Portugal were in full compliance, whereas approximately 88.4% (168/190) of the Spanish farmers showed to be in compliance with refuge requirements (Monsanto Europe S.A., 2014). It has been shown in literature that failure of the technology in multiple cases can be brought back to poor farmer compliance with refuge implementation (Sumerford *et al.*, 2013). In this regard, current levels of structured refuge compliance in Spain are sufficiently high to support sustainability of MON 810. Finally, European farmers have the tendency to rotate plantings in their fields[6]. This is an additional IPM management practice to prevent resistance to develop.

These data highlight the major differences between the EU and the US maize-growing regions. These contrasting differences in farming practices, resulting in a much lower potential of risk resistance development, would suggest that an appropriate proportion of non-*Bt* maize refuge for the EU could be less than the 20% currently used in the US Corn Belt. Therefore, EFSA's recommended sampling frequency of target pests[7] is not justified, and we remain of the opinion that the sampling strategy currently implemented (biennial as long as MON 810 adoption is below or at 80%, with 20% structured refuge at a 5% allele frequency detection level) is sufficient and proportionate to monitor the potential resistance evolution of the target pests.

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[6] *E.g.*, In the EU-28 in 2010 70% of the arable land was found on holdings for which all the arable land was included in planned crop rotation: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Agri-environmental_indicator_-_soil_cover – Accessed 17 November 2014.

[7] See Table 1 in EFSA (2013).

**Table 1:** Comparison of farm numbers and commitment of agricultural land for leading maize producing areas of the EU and the US

| | Number of farms (× 1,000)[1] | Total land area (1,000 ha)[2] | % land of agriculture[3] |
|---|---|---|---|
| **Europe:** | | | |
| France | 516 | 54,397 | 51 |
| Italy | 1,620 | 30,134 | 43 |
| Hungary | 577 | 9,202 | 51 |
| Germany | 299 | 35,713 | 47 |
| Romania | 3,859 | 23,839 | 56 |
| Spain | 990 | 49,851 | 48 |
| Portugal | 305 | 8,909 | 41 |
| Slovakia | 25 | 4,904 | 39 |
| Czech Republic | 23 | 7,887 | 44 |
| **US:** | | | |
| Nebraska | 50 | 20,034 | 92 |
| Iowa | 89 | 14,574 | 81 |
| Minnesota | 74 | 21,852 | 42 |
| Illinois | 75 | 14,592 | 70 |
| Indiana | 59 | 9,371 | 63 |

[1] EU data: European Commission Eurostat: Agricultural holdings (2010); http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/File:Agricultural_holdings,_2000%E2%80%9310_YB14.png – Accessed on 3 November 2014.
US data: USDA National Agricultural Statistics Service (2013); http://quickstats.nass.usda.gov/ – Accessed on 3 November 2014.

[2] EU data: European Commission Eurostat: Land cover overview (2012); http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=lan_lcv_ovw&lang=en – Accessed on 3 November 2014.
US data: USDA National Agricultural Statistics Service (2013); http://quickstats.nass.usda.gov – Accessed on 3 November 2014.

[3] EU data: European Commission Eurostat: Land use: number of farms and areas of different crops by type of farming (2010); http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=ef_oluft&lang=en – Accessed on 3 November 2014.
US data: USDA National Agricultural Statistics Service (2013); http://quickstats.nass.usda.gov – Accessed on 3 November 2014.

**Table 2**: Summary of crop diversity for maize-producing areas of the EU and the US (1,000 ha)

| | Total Ag. Land Area* | Maize (all) | Beans, Peas, Soy | Oats | Wheat (all) | Hay (all) | Barley | Rye | Oilseed Rape, Canola | Sunflower | Sugar Beet | Potato | Grape | Citrus | Olives | Fruit trees |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| France | 27,837 | 3,329 | 100 | 94 | 5,319 | - | 1,636 | 33 | 1,430 | 771 | 394 | 161 | 761 | 4 | 34 | N/A |
| Italy | 12,856 | 1,088 | 224 | 95 | 1,889 | - | 213 | N/A | 18 | 128 | 41 | 54 | 702 | 164 | 1,136 | N/A |
| Hungary | 4,686 | 1,345 | 56 | 51 | 1,091 | - | 262 | N/A | 196 | 597 | 19 | 21 | 72 | N/app | N/app | N/A |
| Germany | 16,704 | 2,500 | 10 | 132 | 3,128 | - | 1,570 | N/A | N/app | 22 | 357 | 243 | 100 | N/app | N/app | N/app |
| Romania | 13,306 | 2,572 | 76 | 182 | 2,098 | - | 495 | N/A | 267 | 1,072 | 28 | 208 | 177 | N/A | N/A | 139 |
| Spain | 23,753 | 550 | 23 | 445 | 2,125 | - | 2,784 | N/A | 39 | 866 | 32 | 72 | 946 | 306 | 2,407 | N/A |
| Portugal | 3,668 | 190 | 2 | 49 | N/A | - | N/A | N/A | N/A | 18 | 0 | 27 | 180 | 20 | 352 | N/A |
| Slovakia | 1,896 | 315 | 30 | 14 | 368 | - | 121 | N/A | 133 | 84 | 20 | 9 | 10 | N/app | N/app | N/A |
| Czech Republic | 3,484 | 331 | 8 | 44 | 829 | - | 349 | N/A | 419 | 21 | 64 | 23 | 16 | N/app | N/app | 17 |
| Nebraska | 18,360 | 4,021 | 1902 | 27 | 497 | 466 | 0.4 | 4 | - | 9 | 14 | 5 | - | - | - | - |
| Iowa | 14,574 | 5,332 | 3696 | 29 | 7 | 514 | 0.4 | 2 | - | - | - | 0.2 | - | - | - | - |
| Minnesota | 9,079 | 3,533 | 2941 | 16 | 499 | 706 | 18 | 4 | 7 | 12 | 180 | 15 | - | - | - | - |
| Illinois | 10,175 | 4,679 | 3651 | 1 | 283 | 52 | 0.4 | 1 | - | - | - | 2 | - | - | - | - |
| Indiana | 5,894 | 2,449 | 2001 | 1 | 158 | 70 | 1 | 0 | - | - | - | 1 | - | - | - | - |

EU data: European Commission Eurostat: Crop products – annual data (2013); http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_cpp_crop&lang=en – Accessed on 3 November 2014.
US data: USDA National Agricultural Statistics Service (2013); http://quickstats.nass.usda.gov – Accessed on 3 November 2014.

* EU data: European Commission Eurostat: Land use: number of farms and areas of different crops by type of farming (2010); http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=ef_oluft&lang=en – Accessed on 3 November 2014.
US data: USDA National Agricultural Statistics Service (2013); http://quickstats.nass.usda.gov – Accessed on 3 November 2014.

N/A = Not Available

N/app = Not applicable

*(iv) Observing resistance allele frequencies above 1% is not sufficient to predict resistance to occur*

The risk of striving for a rapid monitoring turn-around (for example by setting very low allele frequency threshold levels) is the lack of opportunity to replicate the observation over time, *i.e.*, confirm that initial bioassay results are indicative of true resistance or changes in pest susceptibility. Tabashnik *et al.* (2000) reported relatively high frequencies of alleles (*i.e.*, 0.16[8]) affecting resistance to Cry1Ac in pink bollworm populations of Arizona, but this did not correlate with product performance and has not translated into increased frequency of resistant individuals in subsequent generations despite increased usage of *Bt* cotton (Tabashnik *et al.*, 2000). No resistant individuals have been detected via bioassays or PCR-based methodologies since the publication of Tabashnik *et al.* (2000). MON 531 and MON 15985 cottons express high doses of *Bt* proteins for the pink bollworm and resistance has been reported to be inherited in a recessive manner. Possible explanations for not detecting Cry1Ac resistance alleles since 2000 include errors in the initial bioassays, sampling bias during the one year, the adequacy of the structured refuge to delay Cry1Ac resistance in combination with recessive gene action, fitness costs associated with Cry1Ac resistance, and/or the efficacy of the pink bollworm eradication program in the US.

In conclusion, the measurement of resistance allele frequency can only be regarded as one of the elements that inform about potential resistance development. For a high-dose refuge strategy, the limitations of sampling enough genomes within a resistance monitoring program due to low population densities of the pest suggests that changes in susceptibility are more likely to be first observed in the field. However, with sufficient refuge, the rarity of Cry1Ab resistance alleles will allow sufficient time for mitigation of field-control problems. Other best-management practices, such as crop rotation, pyramided *Bt* maize products and other factors to enhance IPM should also be weighed in as additional mitigation measures. Lowering the allele frequency detection limit from 5% to 1% significantly increases the efforts to be undertaken, with hardly any benefit in terms of early resistance development detection.

*(v) In the unlikely event resistance does occur, there is appropriate time to remediate*

In its opinion of 2013, EFSA is concerned that Monsanto's current resistance monitoring strategy will not allow enough time for remediation once problems (decreased susceptibility to MON 810, increased resistance allele frequency, *etc*) are detected. Specifically, if monitoring is designed to detect resistance allele frequency of 5% or greater, EFSA wonders whether there will be enough time to prevent complete resistance across the landscape.

EFSA uses the Alstad and Andow (1995) resistance model from 1995 to track the resistance allele frequency over time (or generations) under various scenarios of MON 810 adoption. Their analysis is less concerned with absolute durability (time until resistance) than with number of generations (or time) it takes for resistance allele frequency to increase from 5% to 50%. If the increase is too fast, EFSA argues that there is not adequate time to respond with a change in IRM strategy, typically an increase in refuge size by decreasing the proportion of hectares planted in the *Bt* product. To better assess the timing of changes in IRM strategy, EFSA considered the time it takes for resistance allele frequency to increase from 1% to 50%, compared to the time it takes to go from 5% to 50% and concluded that detecting allele frequency of 1% might allow adequate time for IRM strategy changes relative to a 5% detection rate.

The modelling effort by EFSA used the Populus software, based on Alstad and Andow (1995), and considered scenarios with MON 810 adoption ranging from 20% to 90%. The modelling effort by EFSA is assuming that resistance is conferred by a single locus with MON 810 providing 99.9% efficacy against homozygous susceptible individuals, 98.9% efficacy against heterozygous individuals and is 0% effective against homozygous resistant individuals, and that the initial frequency of resistance allele R in the population is 0.006. Although EFSA's analysis provided estimates of durability under various scenarios, the primary goal of the analysis was to estimate the number of generations it takes for the R allele frequency to increase from 5% to 50% (or from 1% to 50%). We point out that this estimate will be a

[8] Note that this value is much higher than the detection limit proposed by Monsanto (0.05) in its bioassays with lepidopteran pests in the EU.

function of overall durability, so that if the overall durability of a product is 40 generations, it will generally take longer for the R allele frequency to increase from 5% to 50% than if overall durability was 20 generations. Thus, any estimate should be based on a realistic durability estimate. For example, if a product has been in use for 10 years with no reported cases of resistance, a realistic estimate of durability would be greater than 20 generations (two generations per year).

The simplest models for estimating durability, so-called frequency models, keep track of changes in resistance allele frequencies over time in response to selection, without regard for population sizes. Models such as the Populus model that keep track of population size typically assume that the "natural" mortality rate increases as population size increases, *i.e.*, that there is density-dependent mortality. Because population sizes will be greater in non-*Bt* than in *Bt* fields (at least following *Bt* exposure), the natural mortality rate will be greater in non-*Bt* fields, which has the effect of reducing the productivity of the non-*Bt* refuge. The output of density-dependent models depends on how severe the density-dependent mortality effect is. To illustrate, we adapted a model originally described by Ives and Andow (2002) to estimate MON 810 durability under various assumptions of MON 810 adoption and density-dependent mortality, and using same base assumptions as EFSA's Populus implementation (with the exception of 2nd generation moth preference for *Bt* plants). Different levels of density-dependent mortality were modelled by varying the fecundity parameter F, while holding the density-dependent function fixed at $x^* = \frac{x}{(1+4x)^{0.7}}$, where $x$ is population density after *Bt* selection but before density-dependent mortality is imposed, and $x^*$ is population density after density-dependent mortality is imposed.

Figure 1 below displays model outputs as resistance allele trajectories that increase with generation. In the case of 100% MON 810 adoption (*i.e.*, 80% *Bt* maize plantings and 20% structured refuge), overall durability is 25-30 generations for the cases of no or moderate (F=10) density-dependent mortality, but less than 10 generations for higher (F=50) density-dependent mortality. In the latter case the R-allele frequency increases rapidly from 5% to 50%, in about three generations. It is important, however, to assess how realistic the high density-dependent model is. For example, even in the case of moderate (50%[9]) MON 810 adoption (bottom graph Figure 1); if that model were realistic, resistance should have occurred within 10 years. Because MON 810 has been deployed at moderate adoption for more than 10 years with no indication of resistance, we question the validity of the model with high density-dependent mortality, and instead suggest that model with at most moderate density dependent mortality (blue line in figure) is more realistic. In addition, if population densities have not been high in non-*Bt* fields, the model imposing high density-dependent mortality is probably too conservative, and a more realistic model might impose moderate or even no density-dependent mortality (blue and black lines in figure); with these models, the number of generations between 5% and 50% resistance allele frequency is higher (Figure 1).

Although attaining consensus on the "ideal" model is not the objective, at a minimum we should ensure that models used are consistent with historical observations – which in this case means 10 years or 20 generations of successful product use with no incidence of resistance – and with observed population densities. In other words, we are of the opinion that the interpretation of the modelling performed by EFSA is extremely conservative and should not be construed as an argument to recommend lowering the allele frequency detection limit from 5% to 1% without better congruence with present field observations.

[9] Please note that the definition of 'adoption' in our document differs from the one used by EFSA. Here, 50% MON 810 adoption should be considered as 50% conventional maize cultivation (non-*Bt*), ~41% *Bt* maize cultivation and ~9% structured refuge (non-*Bt*).

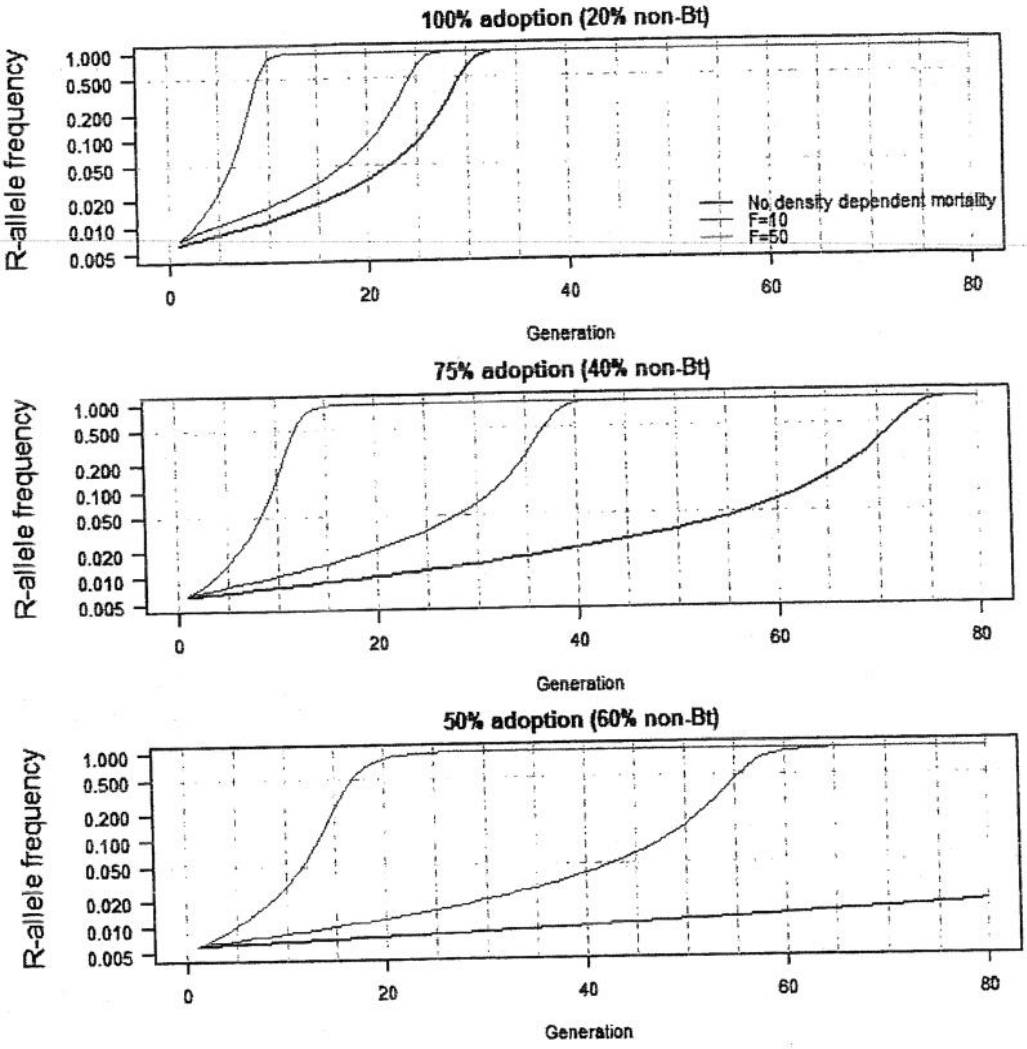


**Figure 1:** Number of generations needed to increase from a 0.5% to a 100% resistant (R) allele frequency considering different MON 810 adoption rates and fecundity parameters (F). Note that y-axis is on log scale. Non-Bt maize should be understood to include both the structured refuge as well as the conventional maize plantings.

### 3. Monitoring geographical 'zones' cannot be put into practice

The concept of a 'hotspot area' was first defined in EFSA's opinion considering Monsanto's monitoring report on MON 810 cultivation during the 2009 growing season (EFSA, 2011) as follows: *a 'hotspot area' is defined as an area of high adoption of maize MON 810 and the presence of multivoltine target pests*. Further (and more specific) clarification on this definition was neither provided in the 2011 opinion, nor in the subsequence one (EFSA, 2012a, 2013).

There are three key elements within this definition, *i.e.*, (i) area, (ii) high adoption of MON 810, and (iii) multivoltine pests. Multivoltine pest is univocally defined, *i.e.*, more than one generation of the target pest per year, and with respect to target pests of MON 810 within the EU, both ECB and MCB are multivoltine in the Mediterranean area. The two remaining elements in the definition, however, are open to interpretation. To come to a workable interpretation, EuropaBio further developed these two variables as follows:

(i) Area = a geographical zone where maize is typically grown following similar agronomic practices isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas, *e.g.*, Ebro Valley;

(ii) The *Bt* maize adoption rate is expressed as a fraction of total maize cultivation in the same area, which is based on official numbers published for this area.

A hotspot (a place with the highest probability of resistance to develop) would then be an area (*e.g.*, Ebro Valley) where MON 810 adoption rate equals or exceeds 80%[10]. These definitions were brought to EFSA's attention by EuropaBio during discussions on the harmonized monitoring plan for the cultivation of GM crops, and more specifically to the European Commission by Monsanto in our letter dated 14 December 2012.

In its most recent opinion, EFSA does not concur with this clear and workable definition of an 'area', and suggests that – to our interpretation – sampling should be concentrated on a 'province' level (Spain), 'region' level (France[11]) or 'lander' level (Germany[11]) (EFSA, 2013). Furthermore, up to three 'zones' of a smaller scale (*i.e.*, county) within each 'area' have to be sampled, based on the MON 810 adoption level expressed in percentage relative to the total arable land.

Firstly, it needs to be repeated that both the ECB and MCB insects can genetically be regarded as one population for the whole of Europe, at least for the places where MON 810 is grown[12]. Many studies have concluded that populations of ECB (Bourguet *et al.*, 2000; Martel *et al.*, 2003) and MCB (Buès *et al.*, 1996; Leniaud *et al.*, 2006), that feed on maize are genetically diverse, and provide evidence of great gene flow, *i.e.*, they can be thought of as one large panmictic population. Other studies of ECB in the US support this conclusion (Kim *et al.*, 2009; Kim *et al.*, 2011; Krumm *et al.*, 2008). Therefore, from a biological perspective there is no reason to subdivide Spain into even smaller areas than is proposed by Monsanto. Further subdivisions will not improve the detection of Cry1Ab resistance for two reasons: (i) there is sufficient gene flow to indicate that monitoring fewer populations will be representative of larger geographic regions, and (ii) also there is sufficient movement of adults to ensure mating between refuge adults and resistant individuals. Therefore, potential larval 'hot-spots' of resistance are not likely to be correlated with 'hot-spots' of mating between resistant individuals, especially with good refuge compliance as is present in the EU.

A second complication that arises with this new recommendation from EFSA is the fact that the definition of 'county' is not harmonized across the EU. Nevertheless, for Spain we could assume that the 'comarcas' could be the county level. An additional and more important issue is that no official numbers can be found for MON 810 or conventional maize plantings for most of the comarcas in Spain. Only data for the comarca level on the four provinces in Catalunya for 2011 were available[13]. Since not all comarcas in Spain have a formal legal status, it is unlikely this information readily exists, which makes the proposed monitoring at this level of detail rather impossible.

Additionally, the sampling effort to be undertaken will increase drastically, only because of the changed definition of an 'area'. The physical and logistical efforts required to comply will not substantially contribute to the reliability of the conclusions around the potential changes in the susceptibility of the pests. Using EuropaBio's definition of an area, three areas were considered (Northeast Iberia, *i.e.*, Ebro Valley; Central Iberia, particularly the province of Albacete; and Southwest Iberia) which were each

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[10] Exceeding 80% MON 810 plantings can occur when a group of farmers individually plant less than 5 ha (and therefore no refuge needs to be planted). Theoretically, their collective MON 810 plantings may exceed 80% of the maize plantings in that area. However, such a patchwork of small MON 810 fields belonging to different farmers is unlikely to be the reality in an area such as the Ebro valley. Alternatively, impaired compliance with refuge implementation requirements can lead to higher than 80% adoption.

[11] It should be noted that MON 810 is banned in France since 2008 and in Germany since 2009.

[12] We understand that for MCB there is a small genetic difference between the Iberian and the Italian/Greece populations of MCB, but the latter are not relevant for the MON 810 monitoring efforts.

[13] http://www.gencat.cat/salut/acsa/html/ca/dir1312/doc16760.html - Accessed 17 November 2014

monitored every second year by taking samples in at least three locations per area. The Ebro Valley for instance consists of the autonomous communities (Comunidades Autónomas) Navarra, Aragon and Catalunya, collectively constituted by eight provinces. If the recommendation from EFSA would become a legal obligation, sampling could[14] then be required in up to 24 'zones' which makes this effort non-proportional and not workable.

Finding appropriate locations within the individual zones/counties/comarcas can be extremely challenging. It needs to be understood that since *Bt* maize products have been cultivated in Spain, the pest pressure has gone down[15]. We also experience this in our current set-up (allowing the detection of a 5% resistance allele frequency), where the collection of sufficient numbers of pest larvae is challenging (Monsanto Europe S.A., 2009, 2010, 2011, 2012, 2013, 2014). If the same number of larvae would have to be collected within each of the 'zones', leave alone to allow for a detection of a 3% or 1% allele frequency, the IRM program would become an impossible task.

A final complication would be that, as EFSA recommends to collect larvae from the refuge areas (or even mix with larvae collected from MON 810 fields), the location of MON 810 fields needs to be known. Monsanto is selling its seeds in Spain through distributors and consequently has access to field level information only in an indirect way. Moreover, Monsanto is certainly not aware, in accordance with EU competition regulations, where the fields of farmers planting MON 810 varieties from other companies are located. Further, now that MON 810 is off patent in the EU, it can be expected that the number of companies marketing MON 810 varieties will increase. This will most likely create additional blind spots on the map if the proposed resolution needs to be obtained.

**Conclusion**

In its opinions on the MON 810 PMEM reports for the 2009-2012 growing seasons EFSA does several recommendations for methodological improvement that would have a substantial impact on the feasibility of its execution. In particular, the refinements proposed by EFSA to the definition of a hotspot, and the aim to detect an occurrence of 1-3% resistance allele frequency in the population of the target pest will require significantly more larvae to be collected and tested via bioassays. The number of locations where samples would need to be collected together with the number of larvae to be collected in each location would significantly increase. In the current letter we elaborated on the reasons why there is no need to change the current IRM plan and also why the improved methodology as proposed by EFSA has negligible added value to the early detection of decreased susceptibility of target insect pests. The long term experience with the MON 810 product, the scientific knowledge of the pest biology, the wealth of EU and global experience showing no resistance development of ECB and MCB towards the Cry1Ab protein, the scientific base for the extremely low likelihood of resistance to develop in these European target pests, the high farmer compliance of the inherently conservative 20% structured refuge requirement, the proven quality of the currently implemented stewardship processes (based on repeated farmer trainings and proper farmer complaint handling systems) and the absence of stringent requirements for similar crop protection applications, together make us conclude that the EFSA recommendations are non-proportional to the likelihood of resistance to develop and urge for keeping the *status quo* in terms of IRM measures to be implemented by the authorization holder.

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[14] Depending on MON 810 adoption per zone (assuming this number is known)

[15] RuralCat, 2013, DossierTècnic N60, p. 7 http://www.ruralcat.net/c/document_library/get_file?uuid=b7e5c9eb-acf4-4367-9d75-9fbe03d9fa4f&groupId=10136 (Figure 6)

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